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OBSERVATIONAL BATTERIES AND MOTOR ACTIVITY

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16. ABSTRACT

The U.S. EPA estimates that there currently are as many as 60,000 chemicals used in this country and that as many as 1000-2000 new ones are introduced into commerce each year. Unfortunately, the ability to assess and predict the possible health hazards associated with exposures has not kept pace with the production of new compounds. As a consequence, there is growing concern that many chemicals introduced originally to improve our lives may in fact be producing a range of organ-related toxicities and disease states. A recent publication of the National Research Countil (1984), for example, estimated that for most chemicals sufficient toxicity information was not available to conduct an adequate health-hazard assessment. As a result the toxic potential of many chemicals is often not appreciated until large numbers of the population have been exposed to them. Under these conditions incidents such as the Ginger Jack poisoning episode in the United States, and the methylmercury poisoning episodes in Japan and Iraq are poignant reminders of the extent to which the nervous system may be at risk for exposure to environmental chemicals. Clearly, then, there is a pressing need for methods suitable for rapidly evaluating the neurotoxic potential of a broad array of chemicals.

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The U.S. EPA estimates that there currently are as many as 60,000 chemicals used in this country and that 1000-2000 new ones are introduced into commerce each year. Unfortunately, the ability to predict and assess the possible health hazards associated with chemical exposures has not kept pace with the production of new compounds. As a consequence, there is growing concern that many chemicals introduced originally to improve our lives may in fact be producing a range of organ-related toxicities and disease states. A recent publication of the National Research Council (1984), for example, estimated that there was insufficient toxicity information available for most chemicals to conduct an adequate health-hazard assessment. As a result the toxic potential of chemicals is often not appreciated until large numbers of the population have been exposed to them. Under these conditions incidents such as the Ginger Jake poisoning episode in the United States, and the methylmercury poisoning episodes in Japan and Iraq are poignant reminders of the extent to which the nervous system may be at risk for exposure to environmental chemicals. Clearly, then, there is a pressing need for methods to rapidly evaluate the neurotoxic potential of a broad array of chemicals.

Screening methods should be sensitive, specific, reliable and efficient. Automated measurements of motor activity ordinarily fulfill these criteria, but it is likely that some behavioral/ neurological effects produced by toxicants may escape detection by relying solely on motor activity measurements. A functional observational battery allows determination of a much broader spectrum of effects through direct experimenter interaction with toxicant-treated animals. The use of functional observational batteries and motor activity in screening for neurotoxic chemicals will be discussed separately in the sections below.

Functional observational batteries. Although functional observational batteries have been used widely in the pharmaceutical industry, to date their use has not been very extensive in neurotoxicology. Currently, however, there is great support for their application. For example, the Office of Toxic Substances (OTS) of the U.S. EPA has prepared a test guideline using a functional observational battery for assessing neurotoxicity (see below). Use of a functional observational battery as a primary screen is also specified along with motor activity in an upcoming publication by the World Health Organization on methods in neurotoxicology. A recent expert panel convened by the Life Sciences Research Organization of the Federation of American Societies for Experimental Biology (FASEB) and commissioned by the U.S. FDA has recommended a similar application. In addition, a functional observational battery is being considered by the Organization for Economic Cooperation and Development (OECD) for inclusion as a screening test.

A variety of functional observational batteries is available. Each represents a collection of non-invasive tests to quantitatively evaluate sensory, motor and autonomic dysfunction in either chemically exposed animals or animals having sustained direct damage to the nervous system. Irwin (1968), for example, has described an extensive series of tests for evaluating the effects of psychoactive compounds in mice (see also Campbell and Richter, 1967). Gad (1982) has introduced a series of tests using rats to screen industrial chemicals for neuromuscular and autonomic toxicity. In addition, Whishaw et al. (1983) have described a broad series of tests for assessing brain lesions in rats. More restricted tests for assessing sensory and/or neuromuscular function in rodents have been described by Jolicoeur et al. (1979), Sterman (1984), Marshall et al.

(1974), Gilbert and Maurissen (1982), Bogo et al. (1981), Meyer et al. (1979), Edwards and Parker (1977), de Medinaceli et al. (1982) and Hruska et al. (1979).

The U.S. EPA has prepared a functional observational battery test guideline which has recently been published (Federal Register, 1985) along with several other guidelines for testing for neurotoxicity. The purpose of the guideline is to standardize observations in order to detect gross functional deficits in animals resulting from chemical exposures. The battery is designed to be used in conjunction with general toxicity testing and/or neuropathological evaluations, and is not intended to provide a detailed account of neurotoxicity. The components of the functional observational battery as specified in the U.S. EPA test guideline are presented in Table 1.

As with any screening effort, many important considerations need to be recognized at the outset (see also Tilson and Mitchell, 1984). First, although the functional observational battery can be applied rapidly, its labor-intensive nature detracts from its efficiency. Second, the observational nature of the battery necessitates incorporating checks on the reliability of measurements, including both between-observer reliability as well as observer reliability over time. Third, techniques need to be applied to efficiently summarize the enormous amounts of data that are ordinarily collected, often using different measurement scales. Inevitably, application of a screening battery involves a compromise between the amount of data that can be collected and the efficiency with which the animals and data can be handled. For this reason, flexibility is always required in order to minimize the number of tests in the battery and yet maximize its predictive power. Finally, although the need for a neurobehavioral

screening battery is now clearly and widely recognized, it is perhaps ironic that none has to date undergone an extensive and coordinated validation process using prototype compounds that produce well-characterized states of intoxication. It is hoped that a workshop such as this one will serve as an impetus to such an undertaking in laboratories throughout the world.

Motor activity. Motor activity is also specified as a primary screening test for neurotoxicity (e.g., Federal Register, 1985). There are many features of motor activity that recommend its inclusion in a primary screening battery. Motor activity is a general feature of the behavioral repertoire of all species and consequently no special training conditions are ordinarily required before exposing animals to chemicals. In addition, motor activity involves the coordinated participation of sensory, motor and integrative systems and therefore it represents a convenient apical test to use, especially when no prior toxicity data are available for a particular chemical.

As with any behavioral test, however, there are several organismic, environmental and experiential factors that may influence the effects of toxicants (for reviews, see Reiter and MacPhail, 1979, 1982). For example, many devices differ in the extent to which they measure locomotor and non-locomotor types of activity. Consequently, in different devices a toxicant might produce different effects depending on whether (1) the compound selectively affected locomotor or non-locomotor types of activity and (2) the device selectively recorded these different forms of activity. For example, acute dose-response functions for the insecticide endosulfan were recently determined using the motor activity of weanling and adult rats tested in either figure-eight mazes or a commercially available

photocell measurement device (MacPhail et al., 1985). The devices differed in terms of size, spatial configuration, photocell orientation and photocell density. The results of this experiment are shown for adult rats in Figure 1. The effects of endosulfan were remarkably similar on motor activity measured in both devices at dosages of 2.5-10 mg/kg. Following the largest dosage, however, motor activity measured in the figure-eight maze was decreased even further whereas in the other photocell device the effect of this dosage did not differ from that of 10 mg/kg. In all likelihood the induction of prominent non-locomotor activities by this rather large dosage (approximately 75% of the LD50) was responsible for the difference in effect produced by endosulfan.

In terms of sensitivity and reliability, automated measures of motor activity are comparable to other behavioral measures in detecting toxicant effects (see, for example, Reiter et al., 1981). Recently, however, there have been trends toward modifying the measurement of motor activity in an effort to enhance its sensitivity and/or increase the amount of information provided in an experiment. Several investigators, for example, have introduced photocell devices involving complex spatial configurations (Ljungberg and Ungerstedt, 1978; Flicker and Geyer, 1982). In addition, Elsner et al. (1979) have used a maze consisting of a series of blind alleys and interconnecting tunnels in which motor activity is recorded by a series of strategically placed photobeams (see also Battig, 1969). The types of data collected include total locomotion, local activity (repetitive interruptions of a single photobeam), duration of occupancy in each area of the maze and the paths taken at each of the intersections. This and related devices offer the possibility of obtaining more detailed information

about the character of motor activity than would otherwise be available if motor activity were measured in a spatially simpler test chamber. However, Stewart (1975) has shown that environmental complexity modified the effect of scopolamine on motor activity. Consequently, it will be important to determine the relationship between toxicant effects on motor activity obtained in devices such as those used by Elsner et al. (1979) and others and those obtained in environmentally less complex devices.

Increasing spatial complexity is a form of environmental challenge that can be applied to toxicant-treated animals. An additional challenge involves placing experimental consequences on the occurrence of motor activity. Gibbins et al. (1968), for example, forced rats to walk on a narrow moving conveyor belt; stepping off the belt resulted in mild electric footshock delivered through side grids. With training, the rats acquired a high degree of proficiency in avoiding shock. Under these conditions, ethanol exposure produced substantial disruptions of behavior and at much smaller dosages than had previously been reported to effect motor activity. An alternative approach was recently described by Kulig et al. (1985) in which rats were forced to locomote in a rotating wheel containing evenly spaced elevated rungs. A microprocessor-based television system was used to study the movement and placement characteristics of one of the rat's hindpaws. Substantial reductions in locomotor ability were obtained following acute exposures to relatively small dosages of diazepam, tremorine, acrylamide and ethanol. For example, the dosages of ethanol required to disrupt locomotion were much smaller than the smallest effective dosage used by Gibbins et al. (1968). Kulig et al. (1985) also studied the effects of subchronic exposure to 2,5-hexanedione. Comparison of control values across the acute experiments or across time in the subchronic experiment revealed a good degree of consistency (coefficients

of variation ranged from 7.5 to 9.1%). Although the approach of placing experimental contingencies on motor activity is not new (see, for example, Meyers, 1965; Slotnick and Jarvik, 1966; Holz et al., 1977; Davis et al., 1973), the data presented suggest that the sensitivity of motor activity measures can be increased by placing environmental demands on toxicant-treated animals. The increase in sensitivity, however, comes about through increasing the time required to train the animals prior to dosing. This, of course, would relegate measures of motor activity to a secondary level in a tier-testing scheme. A particularly intriguing prospect is the possibility that detailed and sensitive automated measures of motor activity may be established that do not appreciably extend the length of time required to test toxicant-treated animals.

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Table 1. U.S. EPA test guidelines on components of the functional observational battery

The following is a minimal list of observations that should be noted:

1. Any unusual responses with respect to body position, activity level, coordination of movement, and gait;

2. Any unusual or bizarre behavior including, but not limited to, headflicking, head searching, compulsive biting or licking, self-mutilation, circling, and walking backwards;

3. The presence of:

   (a) Convulsions;

   (b) Tremors;

   (c) Increased levels of lacrimation and/or red-colored tears;

   (d) Increased levels of salivation;

   (e) Piloerection;

   (f) Pupillary dilation or constriction;

   (g) Unusual respiration (shallow, labored, dyspneic, gasping, and retching) and/or mouth breathing;

   (h) Diarrhea;

   (i) Excessive or diminished urination; and

   (j) Vocalization.

4. Forelimb/hindlimb grip strength.

5. Sensory function. A simple assessment of sensory function (vision, audition, pain perception) should be made.

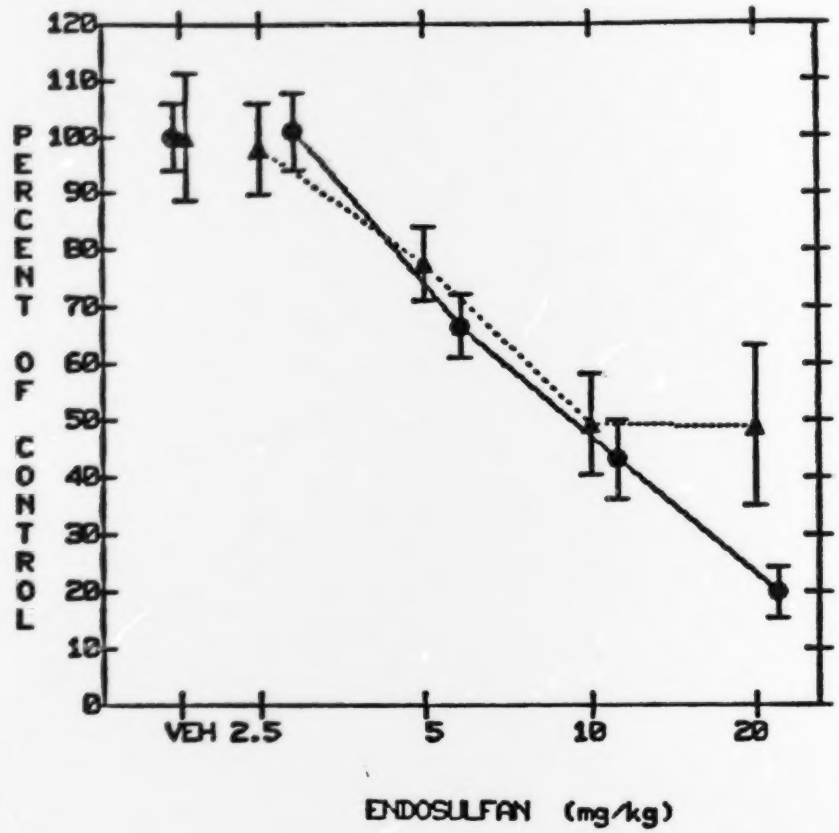

Figure 1. Effects of acute endosulfan administration on the motor activity of adult rats. Circles (●) represent the results for motor activity measured in the figure-eight maze. Rats (N=12 per group) were treated with a dosage of endosulfan or the corn oil vehicle (VEH) and tested for 60 minutes beginning 60 minutes after dosing. Each symbol represents mean ±SEM photocell interruptions for the entire test session expressed as a percentage of vehicle-control data. Triangles (▲) represent the results for motor activity measured in a commercial photocell (Motron) chamber. Rats (N=12 per group) were treated with a dosage of endosulfan or the corn oil vehicle (VEH) and tested for 25 minutes beginning 120 minutes after dosing. Each symbol represents ±SEM photocell interruptions for the entire test session.